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The Catabolite-Sensitive Promoter for the Chloramphenicol Acetyl Transferase Gene Is Preceded by Two Binding Sites for the Catabolite Gene Activator Protein

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DNase I protection experiments have indicated that the cyclic AMP–catabolite gene activator protein complex binds to two regions preceding the chloramphenicol acetyl transferase (*cat*) gene in *Escherichia coli*. One of these lies adjacent to the RNA polymerase binding site, whereas the second lies approximately 130 base pairs upstream from the starting point of transcription. Additional DNase protection experiments and in vitro transcription experiments with modified templates indicate that the catabolite gene activator protein site proximal to the *cat* promoter functions independently of the distal site, indicating that in vitro the second of these sites is not required for transcriptional activation of the *cat* gene.

In *Escherichia coli*, the cyclic AMP (cAMP)-catabolite gene activator protein (CAP) complex acts as a regulator of certain genes or operons, the most notable being the sugar utilization operons, although several other systems have been shown to be under a similar mode of regulation (20). Early studies with the *lac* operon suggested that the cAMP-CAP complex might recognize certain sequences in the vicinity of the RNA polymerase binding site (5), and Gilbert (7) has suggested that transcriptional activation could occur by (i) direct interaction between CAP and RNA polymerase or (ii) cAMP-CAP-induced destabilization of DNA. At present there is no direct evidence to suggest which of these mechanisms prevails, and in fact it may be that both are required. Ebright and Wong (6) have presented strong evidence that direct intercalation between the adenyl moeity of cAMP and a thymine residue within the CAP binding site may destabilize the DNA sufficiently to allow enhanced RNA polymerase interaction. Additionally, chemical and enzymatic probes have indicated that CAP and RNA polymerase occupy adjacent sites on the promoter, as in the *lac* promoter (26), or overlapping sites, as in the *araC* (10, 18), *gal* (27), pBR-P4 (21), and *ompA* (16) promoters, where contact between CAP and RNA polymerase could occur. The promoter for the *araBAD* operon has a CAP site displaced from the RNA polymerase site and contains an additional binding site for the positive regulatory element, *araC* protein (10, 18), and it has been demonstrated that CAP, *araC* protein, and RNA polymerase likewise occupy adjacent sites, allowing possible contact between these three proteins. However, an inconsistency with all of these studies is the position of the CAP site relative to homologous sequences within the promoter (Pribnow box). As the DNA sequences of more CAP sites are elucidated several groups have attempted to construct a generalized consensus sequence serving as a CAP recognition sequence (see below).

Using an in vitro transcription system, we have recently demonstrated catabolite sensitive transcription of the *cat* gene in *E. coli* (11, 12). Concomitant with our research, the DNA sequence of two small transposons carrying this gene was elucidated (1, 14), indicating the presence of a CAP site, very similar in sequence to the CAP site of the *lac* operon, 130 base pairs (bp) upstream from the startpoint of transcription (12). Since a CAP site so far removed from the RNA polymerase binding site appeared inconsistent with present data, we decided to localize the CAP binding site by DNase I protection experiments (24); the results are presented here.

In this study, we demonstrate that the cAMP-CAP complex binds to two regions preceding the *cat* gene. One of these is the site predicted from DNA sequence data, centered some 130 bp from the starting point of transcription. The second site is centered around position −43 and extends

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into the region of the *cat* promoter normally expected to be occupied by RNA polymerase. Binding of the cAMP-CAP complex around position −43 results in a small but highly reproducible alteration in the DNase I cleavage pattern at position −19, indicating that CAP binding may alter the DNA structure within the promoter. Further studies with templates lacking the distal site indicate that catabolite-sensitive transcription, under control of CAP bound around position −43, persists.

In the course of our in vitro transcription experiments, we have also found that transcription of a second RNA species appears to be repressed in the presence of cAMP and CAP, whereas the same system illustrates stimulated transcription of the *cat* gene.

MATERIALS AND METHODS

Plasmids and restriction fragments. The following plasmids (Fig. 1) were isolated by standard equilibrium centrifugation techniques.

(i) pShI 44. pShI 44 was constructed by insertion of a 1.7-kilobase *Pst*I fragment of phage P1 Tn*9* into the unique *Pst*I site of pBR322 (3) and confers resistance to tetracycline and chloramphenicol.

(ii) pH1. pH1 was constructed by in vivo transposition of the 768-bp insertion element IS1 (8) between the two CAP sites of pBR325 (2) and confers resistance to tetracycline, chloramphenicol, and ampicillin.

(iii) pACYC184. Construction of pACYC184 was as previously reported (4).

In vitro transcription experiments were performed on either *Eco*RI-linearized pH1, or the purified 1.5-kilobase *Hin*dIII-*Eco*RI fragment of pACYC184 (Fig. 1). Restriction fragments were purified by electroelution of the appropriate DNA bands isolated by preparative electrophoresis on 4% polyacrylamide gels.

DNase I protection experiments. DNase I "footprinting" was performed as described by Schmitz and Galas (24), with either the 501-bp *Bgl*I-*Taq*I fragment of pShI 44 or an *Hae*III-*Taq*I subfragment, both ^{32}P labeled at the *Taq*I site (Fig. 1). Reaction mixtures (50 μl) contained approximately 0.1 pmol of DNA, 20 μg of CAP per ml, and cAMP at concentrations indicated below; reactions were terminated by the addition of an equal volume of Tris-saturated phenol (50 mM Tris-hydrochloride, pH 8). The aqueous phase was extracted three times with ether and dried of excess ether, and the samples were processed for electrophoresis as described by Maxam and Gilbert (15). Electrophoresis was through 8% polyacrylamide gels containing 8 M urea. To locate the CAP sites more precisely, the same DNA fragment was subjected to the DNA sequencing reaction G + A (15) and run in parallel. Subsequent autoradiography was performed with Kodak X-Omat XAR5 paper and DuPont High-Plus intensification screens.

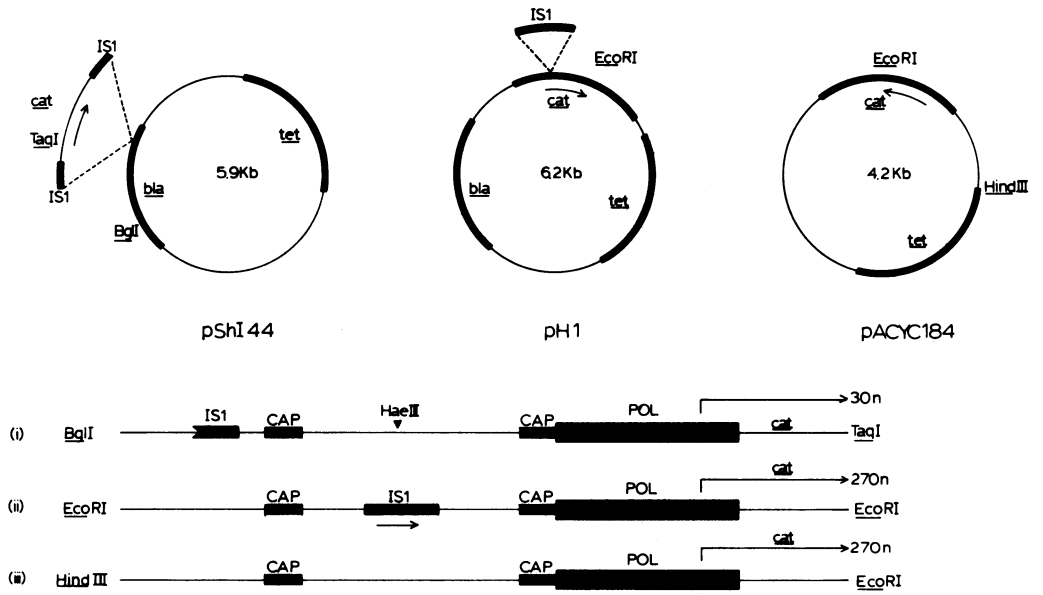

FIG. 1. Plasmids and restriction fragments used. The diagrams are not to scale, and only the restriction sites relevant to the present research are indicated. On each plasmid, the arrow indicates the direction of transcription of the *cat* gene. The lower portion of the figure illustrates restriction fragments used for either in vitro transcription or DNase I protection experiments. (i) The 501-bp *Bgl*I-*Taq*I fragment of pShI 44; the internal *Hae*III site, producing a 100-bp *Hae*III-*Taq*I fragment containing the *cat* promoter and the proximal CAP site, is indicated. (ii) *Eco*RI-linearized pH1; only the *cat* gene portion of the plasmid is indicated here, and the arrow below IS1 indicates the orientation 768-0. Insertion of IS1 is at position 62 of the *cat* gene sequenced by Marcoli et al. (14), and corresponds to position −103 relative to the startpoint of transcription (12). (iii) The 1.5-kilobase *Hin*dIII-*Eco*RI fragment of pACYC184. The size of the run-off transcript from each template is indicated.

In vitro transcription experiments. We have previously reported the protocol for in vitro transcription of the *cat* gene in the presence and absence of cAMP and CAP (11). Reaction mixtures (50 μl) routinely contained 0.1 pmol of DNA, sufficient RNA polymerase to give a polymerase and promoter concentrations of 20 μg of CAP per ml and 200 μM cAMP. For direct autoradiographic visualization of synthesized RNA, reaction mixtures contained 10 μCi of [^{32}P]UTP (400 Ci/mmol) and 10 μM UTP. Fluorographic detection of RNA followed (9), and reactions contained a mixture of 10 μCi of [^{3}H]UTP (43 Ci/mmol) and 10 μM UTP. Reaction products were processed as described above, with the exception that the dried precipitates were resuspended in 0.5 × Tris-borate-EDTA buffer (15) containing 5 M urea and marker dyes. Electrophoresis was through 4% polyacrylamide gels containing 8 M urea.

Materials. Wild-type RNA polymerase was isolated as previously described (11). CAP was a generous gift of J. Krakow. Routine chemicals were purchased from Merck AG, Darmstadt, and electrophoresis reagents were from Serva Ltd., Heidelberg. Radionuclides were from the Radiochemical Centre, Amersham, and restriction enzymes were from Bethesda Research Laboratories, Inc., Rockville, Md.

RESULTS

CAP occupies two sites on the *cat* gene. Our preliminary DNase I protection experiments were performed on a *BglI-TaqI* fragment of pShI 44 containing the initial 195 bp of the *cat* gene of Tn*9* [Fig. 1 (i)]. The cAMP-CAP complex does indeed form a DNase I-resistant complex some 130 bp from the starting point of transcription (Fig. 2a, lanes C and D). We have estimated the size of this CAP site at 22 bp, within which is the region of dyad symmetry GTGA------TCAC, a sequence also found within the CAP site of the *lac* operon (22). The specificity of the interaction is illustrated in a reaction where CAP and DNA were incubated in the absence of cAMP (Fig. 2a, lane B), whereupon the DNase I digestion pattern is identical to the control. Although this result is in good agreement with DNA sequence data (1, 14), suggesting the location of the CAP site on the *cat* gene, Fig. 2b illustrates that the cAMP-CAP complex forms a second DNase-resistant complex very close to the *cat* promoter. This second site is centered around position −43, and the 3′ extremity extends into that region of the *cat* gene we would have expected to be ordinarily occupied by RNA polymerase. We have thus designated this the promoter-proximal CAP site, and that at position −130 as the promoter-distal site. Once again, CAP fails to form a DNase-resistant complex at the proximal site in the absence of cAMP (Fig. 2b, lane

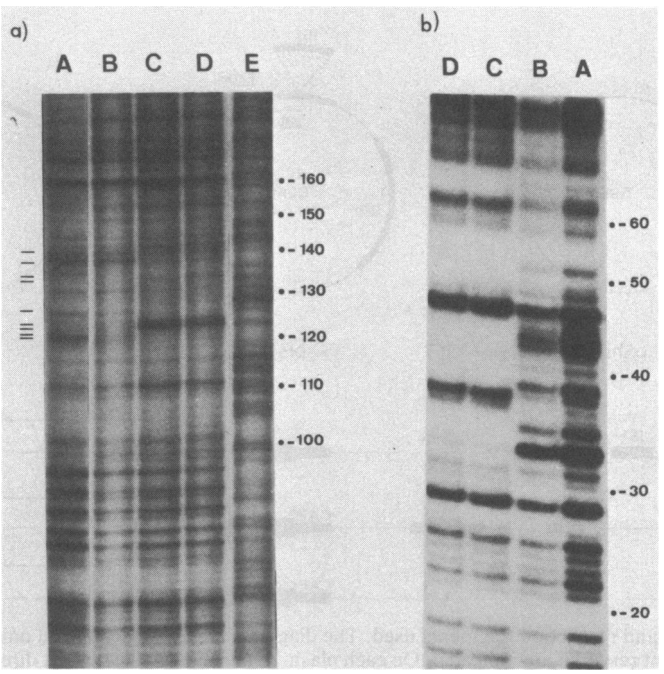

FIG. 2. Binding of the cAMP-CAP complex to the *cat* gene, as demonstrated by DNase I protection. Experiments were performed on the 501-bp *BglI-TaqI* fragment of pShI 44 labeled at the *TaqI* site. (a) Promoter-distal site. Lanes: A, DNA alone; B, DNA plus CAP, no cAMP; C, DNA plus CAP plus 250 μM cAMP; D, DNA plus CAP plus 500 μM cAMP; E, DNA sequence reaction G + A. Since a considerable amount of clarity has been lost on photography, the regions around position −130 displaying altered cleavage have been indicated. (b) Promoter-proximal site. The lane notation follows (a).

B). The size of the proximal CAP site is 20 bp, and experiments with the other DNA strand have not increased the apparent size of the site (data not shown). However, binding of CAP around position −43 appears to cause a small, but highly reproducible, alteration in the DNase cleavage pattern some 15 bp further into the *cat* promoter (Fig. 3, lanes 2 and 3). We do not understand why such an alteration should occur, although an interesting speculation would be that CAP bound in the vicinity of the *cat* promoter is causing a slight alteration in the DNA structure within the promoter. It can also be seen in Fig. 3 that at a cAMP concentration of 5 μM or lower, CAP fails to form a DNase-resistant complex at the proximal site, a result also found with the promoter-distal CAP site (data not shown).

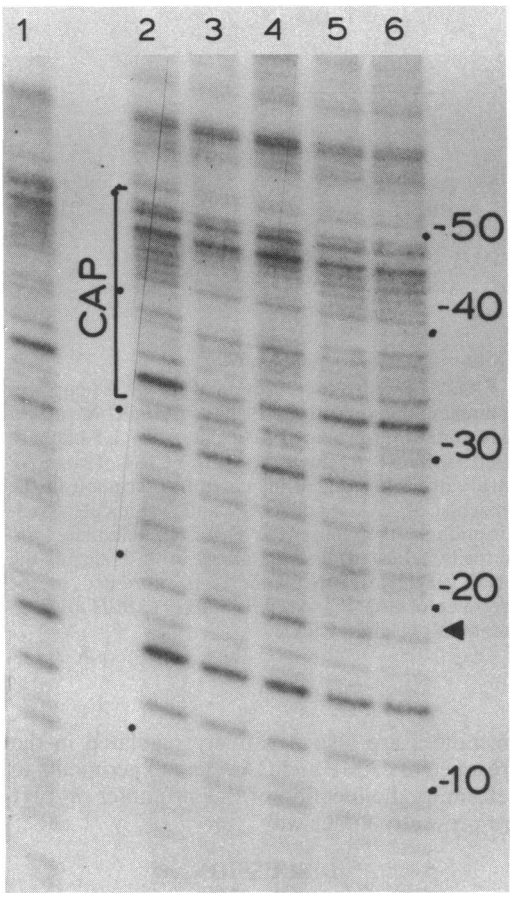

FIG. 3. Effect of cAMP concentration on CAP binding to the promoter-proximal site on the *cat* gene. Indicated here is an enlarged portion of the overall DNase I digestion pattern, indicating only the CAP site and immediately surrounding areas. Lanes: 1, DNA alone; 2, DNA plus CAP, no cAMP; 3, DNA plus CAP plus 250 μM cAMP; 4, DNA plus CAP plus 50 μM cAMP; 5, DNA plus CAP plus 5 μM cAMP; 6, DNA plus CAP plus 0.5 μM cAMP. In lanes 3 and 4, the area further within the *cat* promoter, where altered DNase cleavage was observed in the presence of bound CAP, is indicated by the arrow. It should be noted that lane 4 contained slightly more radioactivity than the others, although the protection pattern was identical to that in lane 3.

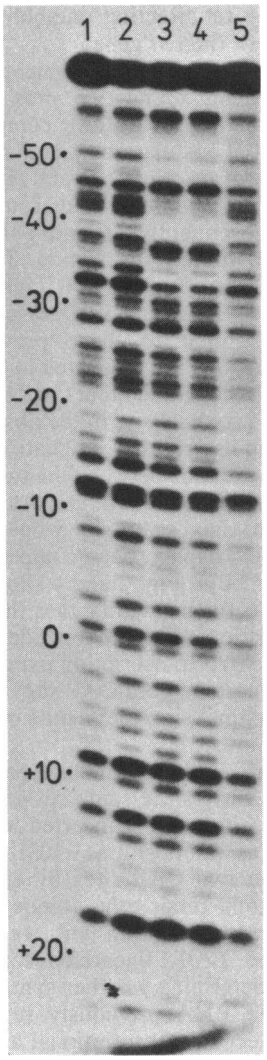

FIG. 4. Binding of CAP to a 100-bp *Hae*III-*Taq*I fragment containing the *cat* promoter and only the proximal CAP site. Lanes: 1, DNA alone; 2, DNA plus CAP, no cAMP; 3, DNA plus CAP plus 500 μM cAMP; 4, DNA plus CAP plus 250 μM cAMP; 5, DNA alone.

As discussed below, these two CAP sites have minimal sequence homology with each other, yet each binds the cAMP-CAP complex equally efficiently. However, as will be shown below, we believe that the promoter-proximal CAP site

alone is the site directing catabolite-sensitive transcription of the *cat* gene.

CAP binds to a restriction fragment lacking the distal site. Having found two sites on the *cat* gene to which the cAMP-CAP complex binds, one of our initial postulations was that both were necessary for transcriptional activation of the gene, i.e., CAP would first bind around position −130 and subsequently around position −43, thereafter activating transcription. To test this, we used a 100-bp *Hae*III-*Taq*I fragment which lacks the distal CAP site in DNase protection experiments similar to those previously described. The results are presented in Fig. 4, from which it can be concluded that CAP binds equally well at the proximal site in the absence of the distal site as in its presence, indicating that there is no interdependence between the two sites. We were concerned that the proximity of the *Hae*III site to the proximal CAP site would somehow destabilize the complex, but a comparison of the results presented in Fig. 2 and 4 shows this not to be true. To further verify that the proximal CAP site functions as an independent unit, we performed in vitro transcription experiments on templates containing both CAP sites or only the promoter-proximal site, the results of which are presented below.

In vitro transcription of modified *cat* templates. Plasmid pH1 carries the same cat gene as pShI 44, but has a copy of IS1 inserted between the two CAP sites (at position −103), effectively separating the two CAP sites by 850 bp. The resulting plasmid retains the unique *Eco*RI site within the *cat* transcription unit; we could thus transcribe the *Eco*RI-linearized form of this plasmid and determine whether synthesis of the 270-nucleotide RNA previously reported (11) was still subject to transcriptional activation in the presence of cAMP and CAP. There appeared to be the same level of enhancement with *Eco*RI-cut pH1 as with the *Hin*dIII-*Eco*RI fragment of pACYC184 (Fig. 5). We have also demonstrated that catabolite sensitive transcription is achieved by using the *Bgl*I-*Taq*I fragment of pShI44, although this transcript appears to be prematurely terminated (12). These results demonstrate that, at least in vitro, the distal CAP site has no effect on transcription under control of the proximal site.

One interesting feature of the data presented in Fig. 5 is that, whereas the presence of cAMP and CAP enhances transcription from the *cat* promoter, transcription from a second promoter appears to be repressed under the same conditions. This transcript, indicated by the arrow, is approximately 100 nucleotides long. The possibility of the cAMP-CAP complex acting as a negative regulator is strengthened by the observations that the *galP2* (17) and *ompA* (13, 16) promoters are also negatively regulated in the presence of cAMP and CAP, and experiments to determine the location of this promoter on pH1 are presently under way.

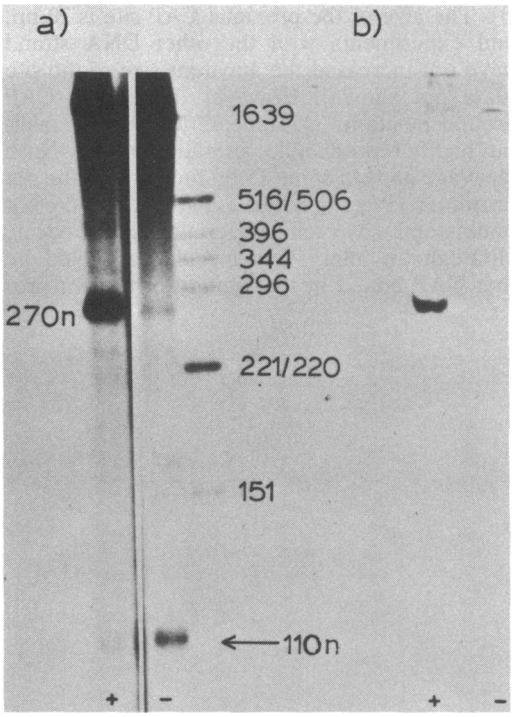

FIG. 5. In vitro transcription of *cat* DNA templates containing one or both CAP sites (a) *Eco*RI-linearized pH1 (promoter-proximal CAP site); (b) 1.5-kilobase *Hin*dIII-*Eco*RI fragment of pACYC184 (two CAP sites). In each case, + indicates transcription in the presence of cAMP and CAP, and − indicates transcription in their absence. The 270-nucleotide transcript is indicated, and the arrow in (a) indicates the RNA species whose synthesis is repressed in the presence of cAMP and CAP. Markers are *Hin*fI fragments of plasmid pBR322.

DISCUSSION

We have established that two regions preceding the *cat* gene in *E. coli* serve as binding sites for the cAMP-CAP complex, namely, a site centered some 130 bp from the transcriptional initiation site (distal site) and a second site centered around position −43 (proximal site) (Fig. 6). Although it was somewhat surprising to find the second of these, our results permit us to compare the DNA sequences within and around both sites with recently compiled data from which consensus sequences have been proposed as indicative of a CAP site.

Before our findings, the DNA sequence of four regions to which the cAMP-CAP complex

binds had been established: *lac* (26), *araBAD araC* (10, 18), and *gal* (27), from which O'Neill et al. (19) derived the consensus 5' AAA$^{G}_{A}$TGTGACA 3'. When we compare the sequences of both CAP sites on the *cat* gene with this, we achieve the following degree of homology:

−141 TACCTGTGACG −131, distal CAP site
5' AAA$^{G}_{A}$TGTGACA 3', consensus (19)
−56 AAAATGAGACG −46, proximal CAP site

i.e., a fit of 7 of 11 for the distal site and 9 of 11 for the proximal site. In light of such a comparison, it is not surprising that CAP binds around position −43, since this site displays greater homology than that around position −130. More recently, however, Queen and Rosenberg (21) have added a new CAP recognition sequence, that of the P4 promoter of pBR322, to the previously known sequences and proposed that the sequence 5' TGTGN$_8$CACA 3' is reasonably well conserved in all CAP sites. If we now make a second comparison, the homology is as follows:

−137 TGTGN$_9$CACT −121, distal CAP site
5' TGTGN$_8$CACA 3' consensus (21)
−52 TGAGN$_{11}$CACG −34 proximal CAP site

Although considerable homology with the repeated sequences can be seen, such homology relationships would require that the distance between the repeated sequences be variable. Further support for the proposal of O'Neill et al. (19) comes from the work of R. H. Ebright (*in* J. Griffin and W. Deux, ed., *Molecular Structure and Biological Function*, in press), who has analyzed the sequences of all known CAP sites and proposed the consensus 5' AANTGTGANNTNNNNCA 3' as a CAP recognition sequence. This consensus takes into account the two CAP sites we have reported here and is very similar at its' 5' end to that of O'Neill et al (19), while extending the sequence in the 3' direction.

In one respect, our data agree very well with all CAP-dependent promoters studied so far in relation to a homologous sequence in the proximity of the CAP binding site. Queen and Rosenberg (21) have noted at 10-nucleotide region of homology, very close to the −35 region of these promoters, with the sequence 5' TTNACACTTT 3'. When we analyze the DNA sequence in the vicinity of the proximal CAP site of the *cat* gene, we find the sequence TTCCAACTTT from positions −26 through −17, which we have overlined in Fig. 6. This does appear to strengthen the proposal (21) that this sequence may serve as an RNA polymerase recognition signal on catabolite-sensitive promoters.

By the biochemical criteria we have imposed (DNase I protection and in vitro transcription) it would appear that the distal CAP site of the *cat* gene need not play a role in transcriptional activation. Were this the case, it has precedents in that nonfunctional CAP sites have been found on both the *lac* (24) and *ara* (10) promoters.

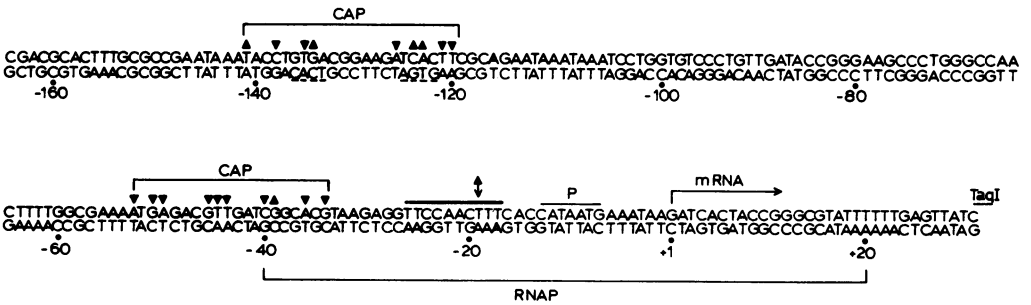

FIG. 6. Summary of DNase I protection data. Presented here are the initial 195 bp of the *cat* gene sequenced by Marcoli et al. (14) with the base pair notation relative to the startpoint of transcription (12). Arrows indicate either protection from (▼) or enhancement of (▲) DNase I cleavage after binding of the cAMP-CAP complex. Abbreviations: P, Pribnow box; RNAP, RNA polymerase binding site. The site further into the *cat* promoter at which altered DNase I cleavage results in CAP binding around position −43 is indicated (↕). Symmetrical sequences within the distal CAP site are indicated, and the sequence within the RNA polymerase binding site, homologous to that proposed by Queen and Rosenberg (21), is overlined.

However, the lack of conclusive in vivo data, such as whether the *cat* gene could be placed under control of the distal site by elimination of the proximal site prevents ruling out the possibility that the distal site is nonfunctional. Experiments to test this are presently underway.

Finally, the promoter-proximal CAP site of the *cat* gene shares a property in common with the *gal* (27), pBR-P4 (21), and *ompA* (16) sites in that it extends into that region of the gene ordinarily occupied by RNA polymerase (25). We have determined both the startpoint of transcription and RNA polymerase binding site of the *cat* gene (12); it appears that CAP and RNA polymerase lie next to each other, indicating that protein-protein contact between these two molecules may be possible.

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LITERATURE CITED

1. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon *Tn*9. Nature (London) **282**:864–869.
2. **Bolivar, F.** 1978. Construction and characterisation of new cloning vehicles III. Derivatives of plasmid pBR322 carrying unique *Eco*RI sites for selection of *Eco*RI-generated recombinant molecules. Gene **4**:121–136.
3. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. G. Crosa, and S. Falkow.** 1977. Construction and characterisation of new cloning vehicles II. A multipurpose cloning system. Gene **2**:95–113.
4. **Chang, A. C. Y., and S. Cohen.** 1978. Construction and characterisation of amplifiable multicopy cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
5. **Dickson, R. C., J. Abelson, P. Johnson, W. S. Reznikoff, and W. M. Barnes.** 1977. Nucleotide sequence changes produced by mutations in the *lac* promoter of *Escherichia coli*. J. Mol. Biol. **111**:65–75.
6. **Ebright, R. H., and J. R. Wong.** 1981. Mechanism for transcriptional activation of cyclic AMP in *Escherichia coli*: entry into DNA to disrupt DNA secondary structure. Proc. Natl. Acad. Sci. U.S.A. **78**:4011–4015.
7. **Gilbert, W.** 1978. Starting and stopping sequences for RNA polymerase, p. 193–205. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Johnsrud, L.** 1979. DNA sequence of the transposable element IS1. Mol. Gen. Genet. **169**:213–218.
9. **Laskey, R. A.** 1980. The use of intensifying screens or organic scintillators for visualising radioactive molecules resolved by gel electrophoresis. Methods Enzymol. **65**:363–371.
10. **Lee, N. L., W. O. Gielgow, and R. G. Wallace.** 1981. Mechanism of *ara*C autoregulation and the domains of two overlapping promoters, P_C and P_{BAD}, in the L-arabinose regulatory region of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:752–756.
11. **Le Grice, S. F. J., and Matzura, H.** 1980. Localisation of the transcription initiation site of the chloramphenicol resistance gene on plasmid pAC184. FEBS Lett. **113**:42–46.
12. **Le Grice, S. F. J., and H. Matzura.** 1981. Binding of RNA polymerase and the catabolite gene activator protein within the *cat* promoter in *Escherichia coli*. J. Mol. Biol. **150**:185–196.
13. **Mallick, U., and P. Herrlich.** 1979. Regulation of synthesis of a major outer membrane protein: cyclic AMP represses *Escherichia coli* protein III synthesis. Proc. Natl. Acad. Sci. U.S.A. **76**:5520–5523.
14. **Marcoli, R., S. Iida, and T. A. Bickle.** 1980. The DNA sequence of an IS1 flanked transposon coding for resistance to chloramphenicol and fusidic acid. FEBS Lett. **110**:11–14.
15. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labelled DNA with base-specific chemical cleavage. Methods Enzymol. **65**:499–560.
16. **Morva, R. N., P. Green, K. Nakamura, and M. Inouye.** 1981. Interaction of cAMP receptor protein with the *ompA* gene, a gene for a major outer membrane protein of *Escherichia coli*. FEBS Lett. **128**:186–190.
17. **Musso, R. E., R. Di Lauro, S. Adhya, and B. deCrombrugghe.** 1977. Dual control for transcription of the galactose operon by cyclic AMP and its receptor protein at two interspersed promoters. Cell **12**:847–854.
18. **Ogden, D., D. Haggerty, C. M. Stoner, D. Colodrubetz, and R. Schleif.** 1981. The *E. coli* L-aribinose operon: binding sites of the regulatory proteins and a mechanism of positive and negative regulation. Proc. Natl. Acad. Sci. U.S.A. **78**:3346–3350.
19. **O'Neill, M. C., K. Amass, and B. deCrombrugghe.** 1981. Molecular model of the DNA interaction site for the cyclic AMP receptor protein. Proc. Natl. Acad. Sci. U.S.A. **78**:2213–2217.
20. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5′ monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**:527–551.
21. **Queen, C., and M. Rosenberg.** 1981. A promoter of pBR322 activated by cAMP receptor protein. Nucleic Acids Res. **9**:3365–3377.
22. **Reznikoff, W. S., and J. N. Abelson.** 1978. The *lac* promoter, p. 221–243. *In* J. Miller and W. Reznikoff (ed), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Schmitz, A.** 1981. Cyclic AMP receptor protein interacts with lactose operator DNA. Nucleic Acids Res. **9**:277–292.
24. **Schmitz, A., and D. Galas.** 1979. The interaction of RNA polymerase and *lac* repressor with the *lac* control region. Nucleic Acids Res. **9**:111–137.
25. **Siebenlist, U., R. B. Simpson, and W. Gilbert.** 1980. *E. coli* RNA polymerase interacts homologously with two different promoters. Cell **20**:269–281.
26. **Simpson, R. B.** 1980. Interaction of the cyclic AMP receptor protein with the *lac* promoter. Nucleic Acids Res. **8**:759–766.
27. **Taniguchi, T., M. O'Neill, and B. deCrombrugghe.** 1979. Interaction site of *Escherichia coli* cyclic AMP receptor protein on DNA of palactose operon promoters. Proc. Natl. Acad. Sci. U.S.A. **76**:5090–5094.